

The effect of acute aerobic exercise on pulse wave velocity and oxidative stress following postprandial hypertriglyceridemia in healthy men

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Abstract Oxidative stress is postulated to be responsible for the postprandial impairments in vascular function. The purpose of this study was to measure pulse wave velocity (PWV) and markers of postprandial oxidative stress before and after an acute bout of moderate exercise. Ten trained male subjects (age 21.5 ± 2.5 years, VO_2 max 58.5 ± 7.1 ml kg^{-1} min^{-1}) participated in a randomised crossover design: (1) high-fat meal alone (2) high-fat meal followed 2 h later by a bout of 1 h moderate (60% max HR) exercise. PWV was examined at baseline, 1, 2, 3, and 4 h postprandially. Blood Lipid hydroperoxides (LOOHs), Superoxide dismutase (SOD) and other biochemical markers were measured. PWV increased at 1 h (6.49 ± 2.1 m s^{-1}), 2 h (6.94 ± 2.4 m s^{-1}), 3 h (7.25 ± 2.1 m s^{-1}) and 4 h (7.41 ± 2.5 m s^{-1}) respectively, in the control trial ($P < 0.05$). There was no change in PWV at 3 h (5.36 ± 1.1 m s^{-1}) or 4 h (5.95 ± 2.3 m s^{-1}) post ingestion in the exercise trial ($P > 0.05$). LOOH levels decreased at 3 h post ingestion in the exercise trial compared to levels at 3 h ($P < 0.05$) in the control trial. SOD levels were lower at

3 h post ingestion in the control trial compared to 3 h in the exercise trial (0.52 ± 0.05 vs. 0.41 ± 0.1 units μl^{-1} ; $P < 0.05$). These findings suggest that a single session of aerobic exercise can ameliorate the postprandial impairments in arterial function by possibly reducing oxidative stress levels.

Keywords Exercise · Postprandial lipaemia · Oxidative stress · Vascular function · Lipid hydroperoxides

Abbreviations

CAD	Coronary artery disease
CE	Cholesterol esterase
CO	Cholesterol oxidase
CRP	C-reactive protein
CV	Co-efficient of variation
ECG	Electrocardiograph
HDL-C	High density lipoprotein cholesterol
HSD	Honestly significant difference
LDL-C	Low-density cholesterol
K ₃ EDTA	Potassium ethylenediaminetetraacetic acid
LOOH	Lipid hydroperoxides
NO	Nitric oxide
NO ₂	Nitrite
NO ₃	Nitrate
NOS	Nitric oxide synthase
eNOS	Endothelial NOS
NO _x	Nitrate/nitrite
ONOO ⁻	Peroxonitrite
O ₂ ⁻	Superoxide anion
PHTG	Postprandial hypertriglyceridemia
PWV	Pulse wave velocity
ROS	Reactive oxygen species

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SOD	Superoxide dismutase
TAG	Triacylglycerols

Introduction

Recent advances have shown that abnormalities in the period after meal ingestion (the postprandial state) are important contributing factors in the development of atherosclerosis (Ceriello et al. 2002). Hypertriglyceridemia (HTG) is now considered to be a risk factor for cardiovascular disease (Bae et al. 2001). It has been proposed that postprandial hypertriglyceridemia (PHTG) can cause endothelial dysfunction, and that repeated episodes of PHTG may promote the development of atherosclerosis (Gaenger et al. 2001).

The term ‘endothelial dysfunction’ relates to an impairment of endothelium-dependent vasodilation (Kalinoski and Malinski 2004). This is characterised by a reduction in the bioavailability of endothelial-derived nitric oxide (NO) in the vessel wall (Bonetti et al. 2003). Vasodilation is perhaps the most documented activity of NO in the cardiovascular system; with research showing that endogenous NO production is involved in the regulation of local vasomotion and blood pressure (Gewaltig and Kojda 2002). Studies have shown that endothelial function and vascular (arterial) stiffness are causally associated and conceptually related (Safar et al. 2001). It is postulated that a decrease in endothelial-derived NO bioavailability may be the critical factor in linking endothelial dysfunction and arterial stiffening (Kinlay et al. 2001).

Cellular respiration in an oxygen-rich environment generates abundant derivatives of partially reduced oxygen, collectively termed reactive oxygen species (ROS) (Leopold and Loscalzo 2005). The role of ROS signalling is currently of great biomedical interest as *oxidative stress* can develop when the capacity of the cell to detoxify these potentially injurious compounds is surpassed (Heistad 2006). An increasing body of evidence suggests that oxidative stress is implicated in the pathogenesis of cardiovascular disease, including atherosclerosis, hypertension and diabetes (Cai and Harrison 2000; Fukai et al. 2002; Stocker and Keaney Jr 2004). As free radicals have the potential to react in an indiscriminate manner leading to the damage of a host of cellular components, an extensive range of antioxidant defences are present to protect from ROS induced damage (Young and Woodside 2001). This range of active antioxidants in the human body includes enzymatic (endogenous) and non-enzymatic (exogenous; required from diet) antioxidants (Finaud et al. 2006).

It has been postulated that PHTG may lead to endothelial dysfunction via an oxidative stress mechanism (Bae et al. 2001; Ceriello et al. 2002). Research evidence suggests that endothelial dysfunction may be precipitated by a rapid inactivation of nitric oxide via oxidant stress caused by ROS. The process may involve the over-production of the superoxide anion (O_2^-), which in turn can inactivate nitric oxide (Roberts et al. 2000), giving rise to large concentrations of peroxynitrite ($ONOO^-$), a potent long-lived oxidant (Cai and Harrison 2000). The subsequent decrease in nitric oxide bioavailability may have negative implications on the regulation of vascular tone.

A growing body of evidence has evolved suggesting that aerobic exercise promotes the removal of triacylglycerols (TAG's) after a high-fat load, bolstered by the observations that endurance-trained men exhibit reduced levels of PHTG and enhanced rates of TAG clearance (Gill et al. 2004). It has also been reported that moderate-intensity exercise ameliorates the endothelial dysfunction induced by the ingestion of a high-fat meal (Padilla et al. 2006). However, to date there appears to be a distinct lack of research investigating PHTG, subsequent oxidative stress production, pulse wave velocity (PWV) (as a marker of vascular function) and the potential benefits of exercise intervention. Thus, the aim of this study was to measure and correlate PWV and markers of postprandial oxidative stress prior to and following an acute bout of moderate intensity aerobic exercise.

Methodology

Subject characteristics

Following ethical approval from the University's Ethical committee and in accordance with the Declaration of Helsinki (1964), 10 ($n = 10$) recreationally trained (participated in at least 2-h week⁻¹ individual or team sport) male subjects (mean VO_2 max 58.5 ± 7.1 ml kg⁻¹ min⁻¹) were recruited from the local population. Before participation all subjects completed a Health History Questionnaire to ensure that they had no medical ailments that would compromise their participation. All participants were non-smokers and were not taking any antioxidant or lipid-lowering supplements. Complete study details including potential risks were fully explained to participants before informed consent was obtained.

Anthropometric measures

Upon arrival at the laboratory, measurements for body mass and stature were taken from each of the subjects. Stature was recorded using a freestanding stadiometer

(Holtain Limited, Great Britain) where, mass was recorded using scales (Seca delta, Germany).

Experimental design

Subjects participated in a randomised crossover study. Where (i) subjects ingested a high-fat meal alone (control), and (ii) ingested the same high-fat meal, followed 2 h later by 1 h of moderate intensity exercise. Trials were separated by no longer than 7 days (the minimum time between trials was 6 days) and subjects were randomly allocated to either the control or exercise trial for the first test and then switched to the other trial group for the second test, 7 days later. Subjects were asked to refrain from exercise and alcohol consumption for 48 h before each trial, and to record and maintain their dietary pattern for the week before the first trial and the week between trials. Subjects arrived at the laboratory following a standard 12 h overnight fast. Subjects, in all but one case, were tested at the same time of day for both trials.

High-fat meal

After baseline measures were taken, subjects consumed the test meal, which consisted of brown bread, butter, cheese, mayonnaise, cooked bacon, and cooked sausages. The content of the meal was calculated according to body mass, with each subject receiving 1.02 g of fat (69% energy content), 0.41 g of carbohydrate (12% energy content) and 0.61 g of protein (19% energy content) per kg of body mass. This represents an intake of 55.4 kJ of energy per kg of body mass (this would represent an intake of 3,881 kJ for a subject of 70 kg). After baseline PWV and venous blood samples were taken, subjects were asked to consume the test meal within a 15-min period. Fluid intake was limited to water (500 ml with meal), which subjects drank ad libitum.

Exercise protocol

For the exercise trial, subjects exercised for 1 h on a motorised treadmill (Power, H-P Cosmos, Germany) at 60% of their maximum heart rate, as determined by a standard test for maximum oxygen (O_2) consumption. Speed was adjusted accordingly during experimental trials to ensure that heart rate values were kept constant (i.e. 60% max HR). Heart rates were continuously measured using an electrocardiograph (ECG) short-range telemetry system (Polar Electro, Finland). Perceived rates of exertion were measured continuously using the Borg scale (Borg 1973). Post-exercise blood samples were corrected for plasma volume shifts using the method of Dill and Costill (1974).

Pulse wave velocity (PWV) measurement

PWV was measured at various time points; baseline, one, two, three (PWV was recorded within 10 min following completion of the exercise bout in the exercise trial), and 4 h post meal ingestion, during the experimental trials. Upon arrival at the lab, subjects were required to rest in a supine position for approximately 5 min. Following this the resting (baseline) PWV of each subject was measured using a sensor-based PWV device (Sensor Technology and Devices Limited, Northern Ireland) as detailed by Mc Laughlin et al. (2003). Pulse wave traces were calculated using the Labview program (Version 7.0). PWV was calculated ($PWV (m s^{-1}) = l/\Delta t$, where l is the distance between the two sensors and Δt corresponds to the time delay in the pulse transit time between the two sensors) following a 10 s measuring period, which was operator-initiated. PWV was measured three times for each individual subject and the mean value ($m s^{-1}$) was recorded. PWV recordings were measured on the same (left) arm for all subjects, between the brachial and radial pulse sites of the arterial tree (these were identified by manual palpation) by the attachment of two PVDF piezoelectric conformal and flexible sensor strips of dimension 2 cm \times 5 cm. The sites were marked for each trial. The distance between the sites was measured for each subject, using a measuring tape, for both the control and exercise trials. The PWV value is based upon the time delay in the mean foot-to-foot, peak-to-peak and cross-correlational waves recorded between the two marked sites in the 10-s measuring period. All tests were conducted between the hours 08.00 and 15.00 to help control circadian variation. Co-efficients of variation (CV) were 2.8% for PWV.

Blood biochemistry

Blood sampling Blood samples were taken at baseline, two, three (in the exercise trial samples were taken within 5 min of completing the exercise bout) and 4 h post ingestion. Samples of blood were obtained, with minimal stasis by extracting blood from a prominent forearm vein, while subjects rested in a supine position. After immediate blood collection, antithrombotic vacutainers containing potassium ethylenediaminetetraacetic acid (K_3 EDTA) and Lithium heparin were placed on ice, whilst the serum separating clot activator tubes (SST) were allowed to clot at room temperature (for 15 min) before centrifugation began at 3,500 rpm for 5 min at 4°C. Plasma and serum were removed and transferred to 1.5 ml plastic vials and were stored at $-70^\circ C$ before subsequent biochemical analysis.

Measurement of superoxide dismutase (SOD) Serum aliquots for SOD determination were assayed at 490 nm on a microplate reader using the Chemicon International, Superoxide Dismutase Activity Assay. The test involves generating O_2^- anions via the addition of Xanthine and Xanthine oxidase solution to the samples. These are detected by a Chromagen solution. When SOD is present, superoxide concentrations are lowered, thereby lowering the colorimetric signals.

Measurement of serum nitrate/nitrite (NO_x) Serum aliquots, for determination of NO_x were assayed for nitrate/nitrite levels using the Nitrate/Nitrite Colorimetric Assay Kit from Cayman Chemical. To determine nitrite (NO_2^-) concentrations, Greiss Reagents 1 and 2 are added to the samples and this results in the formation of a deep purple azo compound. Photometric analysis of this compound then took place. For determination of nitrate (NO_3^-), the samples were added to nitrite reductase before the addition of Greiss reagents. The absorbance of the resulting product was then measured at 550 nm on a microplate reader.

Serum C-reactive protein (CRP) CRP samples were assayed on an Aeroset™ analyser (Abbott Labs, USA) using a CRP high sensitivity assay kit (Randox Laboratories Ltd, Northern Ireland). A 5 ml sample of venous blood was collected into a SST tube and spun at 3,000 rpm at 4°C for 10 min to allow for separation. The sample was combined with a buffer and an anti-CRP coated latex. The formation of the antibody–antigen complex during the reaction results in an increase in turbidity, the extent of which is measured as the amount of light absorbed at 550 nm. CV for CRP were 1.82% at 2.18 mg l⁻¹ and 1.85% at 4.93 mg l⁻¹.

Serum glucose levels Glucose levels were determined by an immobilised enzyme membrane method in conjunction with a Clark electrode on a YSI 2300 analyser (Yellow Springs, USA). The principle of the test involves measuring electrode flow from a steady state H_2O_2 concentration, which is proportional to the concentration of glucose.

Blood lipids Total cholesterol, triacylglycerols and high-density lipoprotein cholesterol (HDL-C) levels were measured by enzyme assay kits, using the Aeroset™ analyser (Abbott Labs, USA). For total cholesterol, samples were centrifuged for 10 min at 3,000 rpm at room temperature. Samples were reacted with cholesterol esterase (CE) and then cholesterol oxidase (CO) resulting in the formation of a chromophore, which was quantitated at 500 nm. For HDL-C, samples were centrifuged at

3,600 rpm for 10 min to separate. A polyanion was added to assist with complexing cholesterol subfractions. The second reagent released only HDL cholesterol allowing it to react with CE and CO, in the presence of chromagen to produce colour. Estimates of LDL-C concentration were calculated using the Friedewald formula (Friedewald et al. 1972). CV was less than 1.6% for triacylglycerols.

Measurement of serum lipid hydroperoxides (LOOH) Serum LOOHs were measured using the FOX-1 assay (Wolff 1994). Standard solutions were incubated for 30 min with the FOX-1 reagent. Preparation of the FOX-1 reagent involves the addition of the following ingredients: 100 μM l⁻¹ Xylenol orange, 250 μM l⁻¹ Ammonium ferrous sulphate, 100 mM l⁻¹ Sorbitol and 25 mM l⁻¹ of sulphuric acid (H_2SO_4). After thawing, 50 μl of each serum aliquot was added to 950 μl of FOX-1 reagent. Samples were then vortexed and left to incubate for 30 min at room temperature in a dark room. The absorbance of the supernatant was then read at 560 nm against the standard curve for the concentration range 0–5 μM l⁻¹. Intra- and inter-assay of CV at 0.57 μM l⁻¹ were 4.6 and 6%, respectively.

Statistical analysis

Statistical analysis was performed using the SPSS social statistics package-version 11.0 (Surrey, UK). Data are expressed as mean \pm SD unless otherwise stated. Data was analysed using a repeated measures two-way analysis of variance (ANOVA), with one between (trial) and one within (time) subject's factor. For a significant interaction effect, within subject factors were analysed using Bonferroni-corrected paired samples *t*-test. Between subject differences were analysed using a one-way ANOVA with a posteriori Tukey Honestly Significant Difference (HSD) test. The linear relationship between two dependent variables was established using Pearson's correlation. The alpha level was established at $P < 0.05$. Prospective calculations of power were performed according to the Altman (Altman 1980) method. Retrospective calculations of power were carried out (using SPSS) for specific variables to determine the suitability of the sample size employed (Fig. 1, 2, 3 and Table 1).

Results

Vascular function

Table 2 indicates that following ingestion of a high-fat test meal, mean PWV increased at 1 h ($P = 0.006$), 2 h ($P = 0.005$), 3 h ($P = 0.0004$) and 4 h ($P = 0.002$)

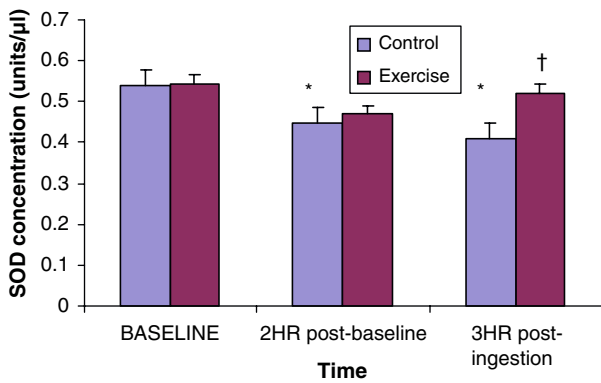


Fig. 1 Superoxide dismutase (SOD) concentration over time * $P \leq 0.05$ versus baseline control; † $P \leq 0.05$ versus 3 h post ingestion control trial

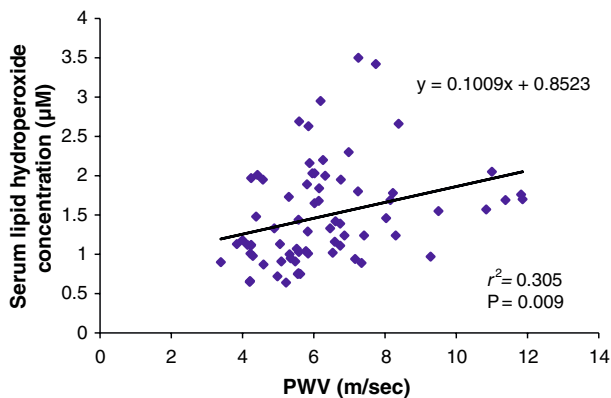


Fig. 2 Correlation of pulse wave velocity (PWV) and lipid hydroperoxides (LOOH)

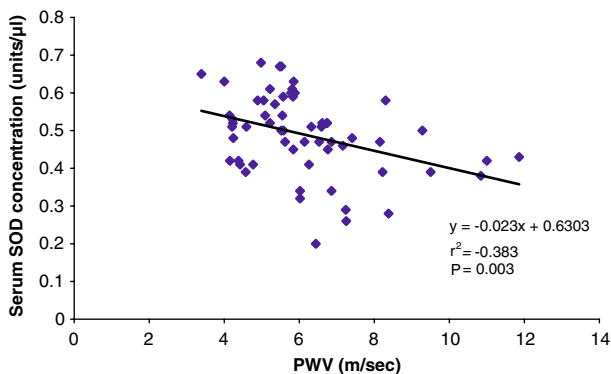


Fig. 3 Correlation of pulse wave velocity (PWV) and superoxide dismutase (SOD)

respectively post ingestion in the control trial ($P < 0.05$). In the exercise trial, increases in mean PWV were observed both 1 h ($P = 0.001$) and 2 h ($P = 0.0001$) post ingestion ($P < 0.05$). However, there were no differences in mean PWV recordings at 3 h (immediately post exercise,

Table 1 Age and physiological characteristics ($n = 10$ subjects)

Dependent variable	Mean \pm SD
Age (years)	21.5 \pm 2.5
Stature (cm)	180.3 \pm 5.6
Body Mass (kg)	77.0 \pm 8.4
BMI (kg m ⁻²)	23.6 \pm 1.6

Table 2 Comparison of PWV following ingestion of high-fat meal

Time	Mean PWV (m s ⁻¹ \pm SD)	
	Control	Exercise
Baseline	5.2 \pm 0.94	4.84 \pm 0.6
1-h post ingestion	6.49 \pm 2.1*	6.99 \pm 2.4†
2-h post ingestion	6.94 \pm 2.4*	7.16 \pm 1.2†
3-h post ingestion	7.25 \pm 2.1*	5.36 \pm 1.1‡
4-h post ingestion	7.41 \pm 2.5*	5.95 \pm 2.3‡

* $P \leq 0.05$ versus baseline control trial

† $P \leq 0.05$ versus baseline exercise trial

‡ $P \leq 0.05$ versus 2-h post ingestion exercise trial

$P = 0.12$) and 4 h (1 h post exercise, $P = 0.19$) post ingestion, when compared to baseline values for the exercise trial ($P > 0.05$). In the exercise trial mean PWV values decreased at 3 and 4 h post ingestion by 25.1% (7.16–5.36 m s⁻¹; $P = 0.006$) and 16.9% (7.16–5.95 m s⁻¹; $P = 0.10$) respectively, when compared to 2 h post ingestion in the same trial. This decrease in mean PWV is not observed in the control trial. Mean PWV increases at 3 and 4 h post ingestion in the control trial by 4.5% (6.94–7.25 m s⁻¹) and 6.7% (6.94–7.41 m s⁻¹) when compared to 2 h post ingestion in the control trial (Table 3).

Oxidative stress indices

Mean serum LOOH levels increased at 2 h post ingestion in the control trial ($P = 0.001$) and remained elevated at 3 h ($P = 0.001$) and 4 h ($P = 0.014$). There were no changes in mean serum LOOH during the exercise trial at 2 h ($P = 0.518$), 3 h ($P = 0.887$) and 4 h ($P = 0.289$) post ingestion. Mean serum LOOH levels decreased at 3 h post ingestion (immediately post exercise) in the exercise trial compared to corresponding serum LOOH levels at 3 h in the control trial (time \times trial interaction, $P = 0.002$).

Antioxidant activity

Serum SOD levels compared to baseline, decreased at 2 h post ingestion; 0.54 \pm 0.1 versus 0.44 \pm 0.1 units μ l⁻¹ and 3 h post ingestion; 0.54 \pm 0.1 versus 0.41 \pm 0.1 units μ l⁻¹;

Table 3 Biochemical markers following ingestion of high-fat meal and exercise

Variable	Baseline	Mean interval (\pm SD)		
		2 h-post ingestion	3 h-post ingestion	4 h-post ingestion
<i>Control</i>				
Total cholesterol (mmol l ⁻¹)	4.26 \pm 0.75	4.15 \pm 0.73	4.2 \pm 0.64	4.28 \pm 0.78
LDL-C (mmol l ⁻¹)	2.58 \pm 0.71	2.17 \pm 0.59	2.08 \pm 0.78	2.24 \pm 0.56
HDL-C (mmol l ⁻¹)	1.28 \pm 0.24	1.26 \pm 0.28	1.18 \pm 0.21* † ‡	1.14 \pm 0.22* † ‡
TAG's (mmol l ⁻¹)	0.81 \pm 0.44	1.54 \pm 0.83*	1.63 \pm 1.05*	1.68 \pm 1.17*
CRP (mg l ⁻¹)	1.19 \pm 1.03	1.17 \pm 1.06	1.26 \pm 1.03	1.19 \pm 1.04
Glucose (mmol l ⁻¹)	5.26 \pm 0.25	4.53 \pm 0.42	4.78 \pm 0.33	5.04 \pm 0.27
LOOH (μ M.L ⁻¹)	1.05 \pm 0.36	1.84 \pm 0.26* ‡	2.15 \pm 0.7* ‡	1.99 \pm 0.78* ‡
NO _x (μ M)	7.3 \pm 2.69	20.89 \pm 13.6	22.88 \pm 15.18	20.95 \pm 15.47
<i>Exercise</i>				
Total cholesterol (mmol l ⁻¹)	4.21 \pm 0.66	4.43 \pm 0.86	4.25 \pm 0.69	4.16 \pm 0.77
LDL-C (mmol l ⁻¹)	2.53 \pm 0.65	2.36 \pm 0.68	2.46 \pm 0.77	2.46 \pm 0.84
HDL-C (mmol l ⁻¹)	1.31 \pm 0.24	1.28 \pm 0.27	1.58 \pm 0.42 †	1.27 \pm 0.26
TAG's (mmol l ⁻¹)	0.81 \pm 0.36	1.7 \pm 0.76 †	1.24 \pm 0.42 † §	1.15 \pm 0.35 † §
CRP (mg l ⁻¹)	1.54 \pm 1.89	1.55 \pm 1.74	1.5 \pm 1.71	1.57 \pm 1.58
Glucose (mmol l ⁻¹)	5.14 \pm 0.35	4.56 \pm 0.38	5.07 \pm 0.12	5.08 \pm 0.10
LOOH (μ M l ⁻¹)	1.13 \pm 0.21	1.23 \pm 0.29	1.16 \pm 0.41 †	1.41 \pm 0.69 †
NO _x (μ M)	7.96 \pm 2.48	20.13 \pm 13	20.76 \pm 13.77	20.53 \pm 13.69

LDL-C low-density lipoprotein cholesterol, HDL high-density lipoprotein cholesterol, TAG's triacylglycerols, CRP C-reactive protein, LOOH lipid hydroperoxides, NO_x total nitrate/nitrite

* $P \leq 0.05$ versus control baseline

† $P \leq 0.05$ versus exercise baseline

‡ $P \leq 0.05$ versus 2-h post ingestion control

§ $P \leq 0.05$ 2-h post ingestion exercise

† $P \leq 0.05$ 3-h post ingestion control

‡ $P \leq 0.05$ versus 3-h post ingestion exercise

in the control trial ($P = 0.001$ and 0.005 , respectively). Serum SOD levels were lower at 3 h post ingestion in the control trial compared to 3 h (immediately post exercise) in the exercise trial; 0.52 ± 0.05 versus 0.41 ± 0.1 units μ l⁻¹ ($P = 0.012$).

CVD risk indices

There were no changes in mean serum total cholesterol ($P = 0.23$), estimated serum LDL-C levels ($P = 0.309$), or serum CRP ($P = 0.75$) either within or between the trials (time \times trial interaction, $P > 0.05$). There were no changes in mean NO_x levels between the trials (time \times trial interaction, $P > 0.05$) but there was a main effect for time for NO_x levels (pooled control and exercise data, $P < 0.001$). In both trials NO_x levels were almost three times greater at 4 h post ingestion than at baseline.

In the control trial mean serum HDL-C levels were lower at 3 h post ingestion (1.31 ± 0.3 vs. 1.22 ± 0.27 ; $P = 0.001$) and 4 h post ingestion (1.31 ± 0.24 vs.

1.18 ± 0.28 ; $P = 0.027$) than at baseline. There were no changes throughout the course of the exercise trial or between trials (time \times trial interaction, $P > 0.05$).

In both trials mean serum triacylglycerols levels increased at 2 h ($P = 0.0025$), 3 h ($P = 0.006$), and 4 h ($P = 0.007$) post ingestion when compared to baseline trial values. However, in the exercise trial mean serum triacylglycerols levels decreased at 3 h (immediately post exercise) (1.7 ± 0.76 vs. 1.24 ± 0.42 ; $P = 0.005$) and at 4 h post ingestion (1 h post exercise) (1.7 ± 0.76 vs. 1.15 ± 0.35 ; $P = 0.004$) when compared to 2 h post ingestion. There were no differences recorded between trials (time \times trial interaction, $P > 0.05$).

Glucose data

There was a main effect for time (pooled control and exercise data, $P = 0.001$). However, there were no changes in mean glucose levels between the trials (time \times trial interaction, $P = 0.19$).

Correlations

There were positive correlations among the changes in PWV and TAG's ($r = 0.226$, $P = 0.04$), and between PWV and LOOH values throughout the trials ($r = 0.305$, $P = 0.009$). However, there were negative correlations observed between brachial-radial PWV and serum SOD levels ($r = -0.383$, $P = 0.003$), and also between serum SOD levels and serum LOOH levels ($r = -0.534$, $P < 0.001$).

Discussion

This study is one of the first to examine the effects of moderate intensity exercise on PWV following the ingestion of a high-fat meal. Mean brachial-radial PWV in the control trial increased significantly up to 4 h following the ingestion of a high-fat meal. This observed increase in PWV was accompanied by, and positively correlated with, a significant increase in LOOH levels (this relationship is quite variable and may not be a robust indicator of causality) and a significant increase in triacylglycerol levels. In addition, a significant decrease in serum SOD levels was also observed postprandially following the ingestion of a high-fat meal alone.

A main finding of this study is that a bout of moderate intensity aerobic exercise attenuated the increase in brachial-radial PWV following the ingestion of a high-fat meal, as observed in the control trial. In both trials PWV increased significantly at 1 and 2 h post ingestion and remained significantly higher at 3 and 4 h postprandially in the control trial. While there were no differences between trials, we are confident that exercise blunted the postprandial increases in PWV demonstrated in the control trial; in the exercise trial mean PWV returned to baseline values at 3 h (immediately post exercise) and 4 h postprandially (1 h post exercise).

The results from the control trial compliment the findings of others (Gaenger et al. 2001, Ceriello et al. 2002, Gill et al. 2004, Blendea et al. 2005) who have shown that the ingestion of a single high-fat meal can significantly impair vascular function. Furthermore, the data suggest an augmented oxidative stress manifested by a decrease in the serum antioxidant enzyme SOD and the concomitant increase in lipid hydroperoxides. Consequently, these findings support the work of others who propose that the postprandial impairment of endothelial function is mediated through an oxidative stress mechanism (Bae et al. 2001, Tsai et al. 2004). Hiramatsu and Arimori (1988) reported an increase in neutrophil-derived superoxide production in patients suffering from HTG and diabetes. These increases in superoxide levels were positively cor-

related with plasma TAG levels. PHTG has also been shown to induce impairments in endothelial function due to an increase in neutrophil-derived ROS, as indicated by augmented plasma LOOH levels (van Oostrom et al. 2003).

Bae et al. (2001) provided more direct evidence, highlighting the relationship between serum triacylglycerol levels, oxidative stress and endothelial function. They reported that leukocyte superoxide anion formation was negatively correlated with endothelial function, and positively associated with serum TAG levels.

In essence, PHTG stimulates leukocytes to produce superoxide anions, possibly via an up-regulation of inflammatory markers such as IL-6 or IL-8, superoxide radicals can then react with endothelium-derived nitric oxide, which results in a decrease in NO bioactivity and the formation of the peroxynitrite anion (ONOO^-) (Fukai et al. 2002). This decrease in NO bioavailability may then be responsible for the observed attenuation in endothelial function.

Although the benefits of exercise are widely known to decrease the risk of cardiovascular disease (Thompson et al. 2003), debate remains over the optimum intensity and duration of exercise that would promote such benefits. Despite this, a growing body of evidence indicates that a single session of moderate intensity exercise can reduce PHTG by ~ 20 to 25% (Gill and Hardman, 2003). A single session of moderate intensity exercise has also been shown to improve postprandial vascular function by significantly decreasing postprandial TAG concentrations (Gill et al. 2004). Furthermore, moderate intensity exercise (60% VO_2 max) has been shown to decrease the levels of ox-LDL, by preventing the ox-LDL-mediated suppression of the antioxidant SOD (Wang et al. 2006). The mechanisms through which exercise could counteract the deleterious effects of a high-fat meal may be multifactorial. First, it is possible for exercise to act through a direct flow mechanism (Padilla et al. 2006). Many studies have investigated the effects of mechanical stimulation of endothelium by shear stress (e.g., exercise training) (Walther et al. 2004). After only 4 weeks of regular physical exercise training, Hambrecht et al. (2003), found that endothelial function was improved in patients with stable coronary artery disease (CAD). In addition, endothelial nitric oxide synthase (eNOS) expression was increased significantly after this period and this could be positively correlated with mean peak blood flow velocity. Therefore, the beneficial effects of exercise on PWV could be mediated via an increase in arterial shear stress which up-regulates eNOS expression and can potentially result in an increase in NO bioavailability. Arterial function, specifically arterial stiffness, is also influenced by changes in transmural pressure (Kinlay et al. 2001) and therefore exercise-induced changes in transmu-

ral pressure may also be responsible for the improvements in mean PWV values following the bout of exercise. However, Wilkinson et al. (2002) have shown improvements in PWV through increased stimulation of NO by exogenous acetylcholine, without concomitant changes in systemic arterial pressure. Moreover, it has also been detailed that improvements in arterial function observed in 12-week-old rats have occurred without any corresponding blood pressure changes (Safar et al. 2001). Improvements in PWV have also been observed after a bout of maximal treadmill running in normal subjects, independent of blood pressure (BP) changes, as there were no differences recorded between pre- and post-exercise BP values. (Naka et al. 2003). We have also shown improvements in brachial-radial PWV in a similar study utilising acute moderate intensity exercise, without any changes in systolic or diastolic blood pressure (unpublished data). The current study only investigated the effects of an acute bout of exercise on PWV and consequently there is a need for more research examining the effects of short term and long-term exercise intervention on PWV.

In the current study a session of moderate intensity aerobic exercise had the effect of decreasing LOOH levels. This may in part be due to an increase in TAG clearance as a result of exercise (Zhang et al. 1998). The decreases in LOOH levels were inversely related to SOD levels. Whereas SOD levels were decreased in the control trial, there were no alterations in the exercise trial. The ameliorating effect of exercise on PWV could thus be due to the decrease in oxidative stress markers. It has been postulated that exercise may perpetuate an increase in ambient SOD levels (Fukai et al. 2002), which could then scavenge the superoxide radical and consequently prevent the decrease in NO bioavailability.

To evaluate this theory, serum NO_x levels were measured in the postprandial phase throughout both trials. NO_x levels reflect the circulating nitrate/nitrite concentrations, which are stable end products of NO (Di Massimo et al. 2004). It has been shown that both exhaustive exercise (Ashton et al. 2003) and moderate intensity training programmes (Maeda et al. 2004) have accentuating effects on NO_x levels. Despite this, there remains a paucity of research examining the effects of acute moderate intensity exercise on circulating NO_x levels. In the present study NO_x levels, somewhat surprisingly, increased at all time points in comparison to baseline values, during both exercise and control trials. However, it must be noted that these increases were not statistically significant. This contradicts the findings of others who have reported decreases in NO_x levels following a high-fat load (Blendea et al. 2005). The increases in NO_x levels observed in each of the trial groups may be accounted for by different mechanisms. In the exercise trial

the increase could be due to the mechanical deformation of endothelial cells perpetrated by the enhancement of blood flow and shear stress associated with exercise, which is largely considered the prime stimulus for the synthesis of endothelial-derived NO (Di Massimo et al. 2004). In contrast, it is theoretically possible that the increase in NO_x levels recorded in the control trial may stem from the interaction of NO and the O_2^- (Loffredo et al. 2006), though this seems unlikely as it has been previously shown that postprandial reductions in NO_x are accompanied by simultaneous increments in tissue nitrotyrosine (Roberts et al. 2000). One other potential explanation for a lack of selective differences for NO_x levels between the groups could be due to the consequence of limited statistical power. Retrospective calculation of power (power = 0.06) shows that for a possible interaction effect to occur, any future study using a similar methodological design would require more subjects. However, it may be possible that the exercise-induced improvements observed in this study may occur independently of alterations in NO levels.

The current study also provided evidence that a bout of moderate intensity exercise attenuated the increase in triacylglycerol levels and the decrease in serum HDL-C levels observed in the control group. In the exercise trial triacylglycerol levels decreased at 3 h (immediately post exercise) and 4 h (1 h post exercise) post ingestion compared to levels at 2 h post ingestion. HDL-C levels were lower at 3 and 4 h post ingestion in the control trial, whereas there were no changes in mean HDL-C levels in the exercise trial. These findings support those of others who have shown that exercise may have a beneficial effect on the triacylglycerol response and HDL metabolism, which may blunt the atherosclerotic process, induced by the high-fat meal (Zhang et al. 1998).

In summary, this study suggests that the PHTG-induced impairment in vascular function can be controlled by a bout of moderate intensity exercise performed 2 h postprandially. It is likely that the exercise-induced improvements are mediated via a decrease in circulating triacylglycerols, which in turn impose a positive change in oxidative stress markers. Markers of NO bioavailability remained unchanged in this study. However, an increase in the NO bioavailability brought about by the increase in shear stress associated with exercise cannot be disregarded. This study highlights the importance of exercise as a potential therapeutic and prophylactic intervention to CVD.

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